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13. ABSTRACT ***(Maximum 200 Words)***

During the fourth year we have focused on making significant progress on all specific aims proposed in the original contract. We have made the following progress in the four hypotheses. Hypothesis I: We have completed most of the data analysis and found that, unlike men, women work their lower legs harder during the latter stages of exercise than during the first hour. We have developed a 3-D volume filling protocol that significantly improved the presentation quality of MRI data. Hypothesis II: We have confirmed that the left biceps depletes glycogen significantly faster in women (both menstrual phases) than in men ($p=0.005$, follicular; $p=0.0184$, luteal). We have refuted our earlier preliminary conclusion, that there is a significant menstrual cycle variation in net liver glycogen depletion rates in our female population. Hypothesis III: We have found a trend toward enhanced muscle glycogen recovery when protein is added to post-exercise carbohydrate supplementation. We believe that this is due to a protein-induced increase in insulin secretion. Hypothesis IV: We have made no significant progress on this hypothesis. We performed 20 total studies this year, averaging almost two per month. This represents an improvement in subject recruitment. We requested and were granted a no cost extension of this contract, and we are currently continuing to perform studies aimed at improving the statistical significance of the data.

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INTRODUCTION

This project was initiated to test four basic hypotheses as they relate to gender. During the past four years we have made significant progress in all areas that were originally proposed and contracted. We have tested the following hypotheses: **Hypothesis I:** ***There are differences in muscle recruitment patterns of men and women when an identical, non-normalized (i.e.-same mass, same duration) repetitive lifting task is performed over a prolonged period.*** This hypothesis (I) has been studied and is near completion. **Hypothesis II:** ***There are gender differences in systemic carbohydrate balance during the performance of this same prolonged repetitive lifting task and during recovery from the task. These differences are the result of women having to work harder to perform the same task. Female menstrual cycle phase may have an effect upon the results.*** This hypothesis (II) has been studied and we have made significant progress; however, this area of study remains incomplete. While we have obtained enough data to draw some basic preliminary conclusions, we need to complete more studies in order to reach a set of final conclusions. We have requested, and have been granted a no-cost extension to continue pursuing this hypothesis. **Hypothesis III:** ***The administration of a carbohydrate supplement immediately before and after the performance of the same prolonged repetitive lifting task may have a glycogen sparing effect, and this effect may be different in men and women.*** We considered excluding this hypothesis (III) form the project last year because the degree of glycogen depletion brought about by our prolonged lifting protocol does not severely deplete glycogen in a specific individual muscle. Rather, the protocol distributes the workload amongst a number of muscles. We decided that this issue is important enough to necessitate the use of another exercise protocol that focuses the workload on a specific muscle or group of muscles. We now employ a bicycling protocol to severely deplete glycogen in m.quadriceps, testing the effect of three different nutritional supplements upon muscle glycogen recovery. Since altering our protocol, we have made significant progress in this portion of the project. **Hypothesis IV:** ***Four consecutive days performance of the same prolonged repetitive lifting task causes an overall***

downward trend in carbohydrate stores. This downward trend is the result of incomplete recovery from each previous day's exercise. The trend may be more pronounced in women than in men. As with hypothesis II, this hypothesis (IV) has been studied and we have made progress, but the study remains incomplete. We have obtained preliminary data and have drawn some basic preliminary conclusions.

BODY OF REPORT

The fourth year of this project has been focused in five major areas: A. Completing the MRI data analysis portion of the project and writing a manuscript **(Hypothesis I)**. B. Completing the data acquisition phase of the first MRS portion of the project **(Hypothesis II)**. C. Making a significant amount of progress on the second MRS portion (4 consecutive days of exercise) of the project **(Hypothesis IV)**. D. Initiating an appropriate exercise protocol to study the effects of nutritional supplementation following exercise **(Hypothesis III)**. E. Improving the effectiveness of our subject recruitment process. In the first major area **(A, Hypothesis I)** we had three goals: 1) Report our data on gender differences in MRI measurement of muscle activity patterns following a single bout of lifting & carrying exercise (the first 15min of the protocol). 2) Complete the analysis of data from the entire exercise protocol (12 bouts of 15min each) and assess the possibility of a progressive increase in muscle activation as exercise proceeds into the final stages of the session. 3) Prepare a manuscript for publication. In the second major area **(B, Hypothesis II)** we had three goals: 4) Complete data acquisition in the first MRS study in 16 male and 16 female subjects (in the females 16 sessions mid-luteal and 16 sessions mid-follicular). 5) Identify differences in physiological response between the male population, the female mid-luteal population, and the female mid-follicular population. 6) Complete MRS data analysis and draw my final conclusions regarding this portion of the project. In the third area **(C, Hypothesis IV)** we had one goal: 7) Complete studies on a significant number of male and female subjects in the 4 consecutive days of exercise study so that a conclusion can be drawn from the data. In the fourth area **(D, Hypothesis III)** we had two goals: 8) Determine an appropriate modification of the

exercise protocol that will allow us to collect relevant data about the effects of nutritional supplementation following severe glycogen depleting exercise. 9) Initiate this final portion of the project and obtain data from a significant number of subjects. In the fifth area **(E)** we had two goals: 10) Increase the number of minority subjects studied. 11) Increase the total number of subjects recruited and improve the recruitment efficiency.

We have successfully completed **area A (goal 1)**, presenting an abstract entitled "MRI of Gender Differences in Exercise" at the 2000 American Physiological Society Intersociety Meeting, The Integrative Biology of Exercise, September 20-23, in Portland, ME. (see Appendix 1a and 1b). In **area A (goal 2)** we have completed the analysis of 80% of the total MRI data that were collected. All data obtained from the female population have been analyzed and about 70% of the data analysis from the male population have been completed. We have analyzed a sufficient amount of data to draw conclusions as to whether the continuation of exercise over 12 15min blocks causes any change in muscle recruitment **(area A, goal 2)**. In other words, as the exercise protocol progresses do some muscles fatigue and other muscles take over to insure that the task is accomplished? We are currently preparing a manuscript **(area A, goal 3)**, and we have further refined our technique for presenting the MRI data (see Figure 4). We anticipate submitting the manuscript **(Hypothesis I)** within the next 2-3 months.

In our second major area of focus **(area B)**, the first goal **(goal 4)** was to complete the data acquisition in the first MRS study in 16 male and 16 female subjects (mid-luteal & mid-follicular). We have continued to make progress on this goal; however, finding suitable test subjects has been an ongoing problem and results in this area have been disappointing. We have now completed 25 total studies (14 M, 6 F mid-luteal, and 5 F mid-follicular). The difficulty in recruiting suitable female subjects persists, and during the past year it has become increasingly difficult to recruit women who are not on birth control. In **area B, goal 5** we have identified gender differences in muscle glycogen depletion in the left biceps brachii, with no significant gender differences observed in the left vastus lateralis or in net liver glycogen depletion. We have analyzed all data **(area B, goal 6)** that have been collected thus

far in the first MRS study **(Hypothesis II)**, and have solidified our initial conclusion that women deplete more available energy stores in their upper arms than men.

We have continued the second MRS study **(area C, goal 7)**; however, we have completed the protocol with only one additional male subject (total of 4 studies, 3M, 1F). Based on our lack of progress in this area, we feel that goal 7 has been unsuccessful. In our last report (year 3) MRS data from the first and second MRS studies led us to conclude that the carbohydrate supplementation protocol **(Hypothesis III, area D)** would not be a worthwhile pursuit. We have reconsidered our conclusion and restructured the experimental protocol to focus glycogen depletion on m.quadriceps **(area D, goal 8)**. While the exercise protocol that we have used throughout this project provides a fair representation of the type of physical challenge that a soldier might be expected to encounter in the field, it does not focus a severe metabolic demand on any specific muscle. It is also clear that lifting and carrying is not the only type of physical challenge that a soldier might be expected to encounter. To test the effect of nutritional supplementation upon muscle glycogen recovery a more focused type of exercise was required; however, we believe that this type of physical challenge (focused work) is also commonly encountered in the field, particularly in combat. We feel that information about the comparative effect on glycogen recovery of different nutritional supplements will valuable, and have re-structured our experimental protocol accordingly. Employing a bicycling exercise protocol we have completed 12 studies in 7 different subjects (all trained cyclists) **(area D, goal 9)**. In the final area of focus **(area E)** we continue to have only limited success. In **area E, goal 10** we have completed 3 studies involving minority (black) subjects (1M, 1F both phases of her menstrual cycle), and we currently have a tentative agreement with two more black subjects (2 F) to participate. As with last year **area E, goal 11** has been unsuccessful, and our efficiency in subject recruitment and retention has remained poor during the past year.

During year four this project has continued to progress; however, the contract is currently not on track with the revised SOW. Again, the primary reason for this is difficulty in recruiting and retaining suitable test subjects. It is our intention to continue with this project until it is completed, and to this end, we have requested and

received a no-cost extension. Despite the difficulties, this project continues to generate clear data that leads us to definite conclusions.

A. Experimental methods, assumptions, and procedures:

With the exception of the revised nutritional study, the experimental methods, assumptions, and procedures have been largely described in previous annual reports (1,2,3). This section focuses primarily on the continuation of experiments that have been described in these earlier reports (1,2,3), with more detail given in the description of the revised nutritional studies.

A. Complete the MRI portion of the project **(Hypothesis I)** – This area is essentially complete. We are currently completing the remainder of data analysis in the male subject population, and the manuscript is in preparation.

1. Report data on gender differences in MRI of single bouts (15min) of repetitive lifting – Data was presented in September at the 2000 APS Intersociety Meeting (Appendix 1a & 1b), successfully completing this goal (see also Figure 1a & 1b and Figure 4).
2. Complete MRI data analysis from the entire exercise protocol and assess the possibility of changes in muscle activation in the latter stages of exercise – We have analyzed 100% of the MRI data from the female subject pool and 70% from the male subject pool. The volume of analyzed data has been sufficient to allow us to draw conclusions about gender differences in muscle activation patterns and how patterns change over the course of prolonged exercise. While some of the data remains to be analyzed, we feel that this goal has been successfully accomplished (see Figure 2 and Figure 3).
3. Prepare a manuscript for publication – A manuscript is currently in preparation. Because the manuscript is not complete, this goal has been partially successful.

B. Complete the data acquisition for the first MRS study **(Hypothesis II)** – This area remains incomplete, due primarily to the difficulty in recruiting appropriate female subjects.

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4. Complete data acquisition on 16 male and 16 female (both mid-luteal and mid-follicular phases) subjects – We have completed 25 total studies, out of 48 total studies proposed (14M, 6 F mid-luteal, and 5 F mid-follicular). This goal has not been successfully accomplished.
5. Identify gender differences and/or menstrual cycle differences in metabolism – We have identified gender differences in muscle glycogen depletion rates in m.biceps brachii (Figure 5b). Glycogen depletion rates in m.vastus lateralis are similar between genders as is net liver glycogen depletion (Figure 5a and Figure 7). While there is a trend toward more rapid glycogen depletion in women in the mid-follicular phase of their menstrual cycle, differences are not significant. This goal has been only partially accomplished.
6. Complete data analysis and draw final conclusions regarding hypothesis II – Data analysis is complete on all studies that have been completed; however, a number of studies remain to be completed. Therefore, our final conclusions are tentative at best and subject to change as more data are acquired. This goal has been partially accomplished.

C. Make a significant amount of progress on the study of 4 consecutive days of exercise **(Hypothesis IV)** – We have been largely unsuccessful in this area of the project.

7. Complete a significant number of studies – We have studied one additional subject in this portion of the project. This area remains the most difficult for recruiting appropriate test subjects; therefore, this goal has been unsuccessful.

D. Initiate an appropriate exercise protocol to study the effect of carbohydrate supplementation following exercise **(Hypothesis III)** – We have made significant progress in this area, the experimental methods, assumptions, and procedures are described below (see Figure 8a & 8b and Figure 9).

Purpose: To determine the effect of combining protein with carbohydrate in a post-exercise supplement, upon the rate on muscle glycogen storage.

Materials and Methods:

Subjects: Eight trained, male, cyclists (ages 18-25 yr) will be recruited for this study

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Protocol: A medical history questionnaire and evaluation will be performed on each subject prior to their participation in the study.

Control Period. Subjects will record their dietary intake for 3 days and maintain a cycle training log during the month before the study begins (i.e., control period). Subjects will perform a peak VO^2 cycle ergometry test to determine their aerobic fitness level, within 2 weeks of beginning the dietary phase of the study. Subjects then will be rank ordered according to their aerobic fitness and randomly assigned to the treatment groups. Temporal trends will be avoided by blocking the treatment conditions to provide sets of three subjects, one subject assigned to each of the three treatments (e.g., B_1,C_2,A_3,; C_4,A_5,B_6:..).

Study Period: After the control period, subjects will report to the Yale Medical School, Clinical Research Center (CRC) on a Friday afternoon. Subjects will eat a normal mixed meal and perform an overnight fast (12 hrs). The following morning each subject will cycle a 65-75% peak VO^2 for 2 hours until blood glucose reaches 3.6 mmol/L (65mg/dL). During the exercise session, subjects will consume 150ml of water every 15 minutes. Following the 2 hour cycle exercise, subjects will consume one of three nutrient supplements [A= 105 g carbohydrate, and 9g fat (501 kcal), B= 147 g carbohydrate and 9g fat (669 kcal), or C= 105g carbohydrate, 42g protein and 9g fat (669 kcal)] immediately after and again 2 hrs post-exercise.

__Measurements:__

Blood Metabolites (insulin, epinephrine, norepinephrine, glucose, lactate, fatty acids).

Blood samples will be drawn prior to exercise and at 90 min during the exercise and at 0, 15, 30, 60, 90, 180, 210 and 240 min after each exercise trial, from a catheter inserted into an anticubital vein. Blood glucose and lactate will be measured after every 5 sprints to document exhaustion. Approximately 5 ml (about one teaspoon) of blood will be removed at the time of each blood draw, or a total of approximately 55 ml during each of the three treatment trials. Prior to, during and every 30 min during the 2-hour exercise trials, heart rate and oxygen consumption will be monitored.

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Muscle Glycogen.

Muscle glycogen will be measured in the thigh muscle (quadriceps) using NMR spectroscopy prior to, immediately (6 min), 15, 30, 45, 60 min, 90 min, 2 hrs, 3 hrs and 4 hrs after each exercise trial. This is a non-invasive procedure, each measurement requires that the subject lie on a padded platform in a MRI coil (1 meter in diameter) for approximately 10 minutes.

This study has been redesigned to severely deplete muscle glycogen in m. quadriceps femoris so that the effect of different nutritional supplements upon muscle glycogen recovery can be compared. This area of the project has been successful.

8. Modify the exercise protocol to provide an appropriate test of the effect of carbohydrate supplementation upon muscle glycogen recovery – We have successfully performed the modified protocol described above, and found it to be a valid test of the effect of nutritional supplementation upon muscle glycogen recovery.
9. Perform this study on a significant number of subjects – We have completed 12 studies in 7 different subjects, including three trials each in the three different conditions described above. This goal has been successfully accomplished.

E. The final major area of focus during the past year has been to improve our performance in the area of subject recruitment. We have had little success in this area over the past year, and we continue to struggle with subject recruitment.

10. Increase the number of minority subjects studied – We have completed 3 studies involving black subjects (1M, 1F in both menstrual phases), and we currently have an agreement to participate with two more black female subjects. Therefore, we have experienced some marginal success with this goal.
11. Increase the total number of subjects recruited and improve the recruitment efficiency – As reported last year, we have again failed to improve on either of these goals.

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C. Results:

1. Completion of the MRI portion of the project **(Hypothesis I)** – We have reported the following results from our comparison of gender differences following a single 15min bout of lift & carry exercise. **Introduction:** During complex movements the human body routinely activates a number of muscles to distribute the total work and enable the performance of a task without significantly challenging individual muscles. How hard a muscle must work to accomplish a given task is directly related to its size. The female musculature is generally smaller than that of males, and as a result, the mean lifting strength in women has been shown to be ~50% of men (4). Because of their smaller body size, women are at a 2X greater risk of injury than men performing identical exercise tasks (5). MRI is known to be a reliable means of detecting muscle activity resulting from complex exercise tasks (6,7). Furthermore, the degree of exercise induced change seen in MR images of a given muscle is a function of how hard that muscle is working relative to its maximum capacity to work (8). The purpose of this study was to compare male and female muscle activation patterns resulting from the performance of an identical complex exercise challenge, lifting and carrying a 65-pound box.

   Methods: Six males (25±3yrs, 85±3kg, 180±3cm) and six females (27±3yrs, 60±3kg*, 165±3cm*, $p \leq 0.005$) repeatedly lifted a 30kg box from floor level, carried it 3m, and placed it at a height of 132cm. Subjects performed 3 lifts/min over a 15min period. T2-weighted transverse MR images (GE Signa 1.5T) were obtained from the whole body before (180 slices) and immediately after (120 slices) exercise. Using a body RF coil 30 slices were acquired at each station (slice thickness=10mm, ISS=0mm, FOV=40X20cm, matrix=128X128). Back-to-back echo-planar MR images were obtained at TE=30msec and TE=60msec. Once a noise-reduction routine (non-linear filter, similar to a top-hat transform) was applied, the raw K-space data were converted to spatial domain images via a Fourier transform process. The reconstruction process was performed in Matlab

(The MathWorks, Inc., Natick, MA). Muscle activation status was determined by computing changes in T2 values for selected muscles between pre- and post-exercise imaging sessions. Fifty-two muscles were assessed for exercise induced T2 increase. Because these T2 increases are transient, images were acquired over the initial 10min period beginning at cessation of exercise.

Results: Of fifty muscles assessed (25 each r & l), female subjects recruited a significantly greater number of different muscles (37±2) than male subjects (27±2) ($p=0.0106$). The exercised induced change in T_2 was greater in females in both the upper and lower body (Figure 1a & 1b). The greatest differences were observed in the upper body. T_2 changes in individual muscles are given in Table 1a & 1b.

Summary: MRI has been used to demonstrate gender differences in muscle activity brought about by a lifting & carrying task. Since the degree of exercise induced change on MRI's is known to be workload dependent (8) this study demonstrates that female subjects had to work harder to accomplish the task than their male counterparts. The female population activated a greater number of muscles and used the active muscles at a higher level than the males (Figure 1a & 1b).

Conclusions: This study was funded by the U.S. Army (DAMD17-96-C-6097), and intended to compare genders during the performance of a standard military lift & carry task. However, these data can be applied to any population that lifts & carries a load as a part of their job, or in other aspects of daily living. We conclude that, because of their smaller stature, women must work harder to accomplish the same task and therefore may be at a greater risk of injury. Furthermore, the muscle activation patterns clearly indicate that the difference in work (male versus female) is distributed throughout both the upper and lower body.

Analysis of MRI time-course data: We have continued to analyze MRI time-course data to assess possible changes in muscle activation patterns over the

course of 12 blocks of 15 minutes of exercise (3hrs total exercise). We have determined that T2 increases are fairly consistent in both male and female populations over the three hours of exercise. All T2 increases (upper and lower body) in the male population were significantly smaller in the three time periods studied (0-1hr, 1-2hr, and 2-3hr of exercise) ($p \leq 0.04$, Figure 2a,b,c and Figure 3a,b,c). While there was a trend toward greater T2 increases in the female population (quadriceps, lower legs & gluteals) during the final hour of exercise, significance was established only in the lower legs (first hour vs third hour, $p=0.0239$) (Figure 3c). T2 increases were stable in the male population over the three hours of exercise (upper and lower body), even declining in the hamstrings during the second hour (first hour vs second hour, $p=0.0403$) (Figure 3b).

We have further refined our method of presenting ΔT2 maps in human subjects, using a 3-D volume filling protocol on our stacked 2-D MRI data. This is another time-consuming process; however, the results are spectacular (Figure 4).

2. Continuing progress on the MRS portion of the project **(Hypothesis II)** – These results remain inconclusive owing to the small number of studies completed to date; however, we have completed 7 more studies. Our initial results have been solidified and there are several interesting trends that point to potentially significant final conclusions. From the 25 studies completed thus far, we have compared 14 male subjects with 6 female subjects in the luteal phase of their menstrual cycle and 5 female subjects in the follicular phase. We have measured glycogen depletion patterns in the left quadriceps muscles (vastus lateralis and vastus intermedius) (Figure 5a and Figure 6a) and in the left biceps brachii (biceps and brachalis) muscles (Figure 5b and Figure 6b). We have also measured net glycogen changes in the liver during the prolonged lift and carry protocol (Figure 7).

    We continue to observe a trend toward higher glycogen depletion rates in the left quadriceps (m.vastus lateralis, m.vastus intermedius) muscles of the male population (-5.4±1.1mmol/l-hr) than in the female population in the luteal phase of the menstrual cycle (-2.3±2.8mmol/l-hr, luteal) (Figure 5a). However,

differences are not significant, and glycogen depletion rates are similar between man and women in the follicular phase (-6.6±2.8mmol/l-hr, follicular) (Figure 5a). With the addition of this year's data, the variability of this measurement suggests that in m.quadriceps we will probably not detect significant differences in glycogen depletion rates between men and women. We compared quadriceps glycogen depletion rates during the first hour of exercise (-6.0±2.5mmol/l-h, male, -7.0 ±4.1mmol/l-hr, luteal and, -10.6±2.4mmol.l-hr follicular) with those during the final two hours (-3.7±3.0mmol/l-hr, male, –1.1 ±4.8mmol/l-hr, luteal and +5.8±5.4mmol/l-hr, follicular). We observed a trend toward less glycogen depletion in both the male and female population during the latter stages of exercise; however, differences were not significant (Figure 6a). Therefore, it does not seem reasonable to predict that this project will reveal significantly higher quadriceps glycogen depletion rates in men than in women, nor will it reveal menstrual cycle differences in an unpaired female population.

In the left biceps muscle we have observed significantly higher glycogen depletion rates in the female population during both menstrual phases (-14.6 ±3.5mmol/l-hr, luteal, p=0.0050) and (–9.9±3.0mmol/l-hr, follicular, p=0.0184) than in the male population (-2.2±1.6mmol/l-hr) (Figure 4b). We compared biceps glycogen depletion rates during the first hour of exercise (-6.5±2.4mmol/l-hr, luteal and, -7.0± 6.4mmol.l-hr follicular) with those during the final two hours (–12.0 ±2.9mmol/l-hr, luteal and -15.4±5.9mmol/l-hr, follicular). This comparison revealed a trend toward greater glycogen depletion in the female population during the latter stages of exercise (Figure 6b). Based upon the variability of the data, it does not seem reasonable to predict that we will see significant differences in early versus late biceps glycogen depletion rates, nor do we expect to detect menstrual cycle differences in an unpaired female population.

There were no differences in net liver glycogen depletion rates between men (-0.146±0.017 mmol/l-min) and women in the follicular phase of their menstrual cycle (-0.149±0.024mmol/l-min) or in the luteal phase (-0.098

±0.015mmol/l-min), nor were there menstrual cycle differences luteal vs follicular (Figure 7). Based on the variability of these measurements it now does not seem reasonable to predict that we will be able to establish either gender differences or menstrual cycle differences in net liver glycogen depletion rates during exercise over the course of this study.

3. Increase the number of studies of 4 consecutive days of exercise **(Hypothesis IV)** - We have no further data to report on this portion of the project. The addition of one study does not change our initial preliminary conclusions. We still believe that we will observe a progressive supercompensation of glycogen when an iso-caloric mixed-meal diet that was administered to each subject. This suggests that a high carbohydrate diet is not required to produce supercompensation with consecutive days of this type of exercise.
4. Nutritional supplementation study **(Hypothesis III)** – We have compared muscle glycogen recovery from severe glycogen depleting exercise in m.vastus lateralis and m.vastus intermedius under three different nutritional conditions. One of three nutritional supplements was administered immediately after cessation of exercise and two hours into the recovery period: Protocol A = 80g of carbohydrate + 28g of protein, total 108g; Protocol B = 108g of carbohydrate; Protocol C = 80g of carbohydrate. All supplements also contained 9g of fat. Results are given in Figure 8a & 8b and in Figure 9. We have studied three subjects each in protocols A, B, & C. In all studies muscle glycogen was significantly depleted (mean±SE glycogen depletion = 112.6±6.0mmol/l) down to 36.5±3.5 mmol/l at the beginning of the recovery period. The time-course of glycogen recovery for protocols A, B, & C is given in Figure 8a. By the end of 240 min recovery glycogen levels were significantly higher in subjects given carbohydrate plus protein that in those given carbohydrate only ($p \leq 0.03$). When glycogen synthesis rates were calculated for the three treatments, we observed that the ergogenic effect of protein occurred mostly in the first hour of recovery (Figure 8b). The rate was greater ($p \leq 0.03$) in the first hour of treatment A (30.6±6.8 mmol/l-hr) than in the first hour of treatment C (6.2±2.1mmol/l-hr)

(Figure 7b). This may be the result of a greater release of plasma insulin when protein is included in the nutritional supplement. Insulin samples were obtained and are currently undergoing analysis. We have blood glucose data that support the idea that protein triggers greater insulin release (Figure 9). During the initial 120min of recovery blood glucose levels were significantly lower ($p \leq 0.04$) in subjects undergoing treatment A than in those undergoing the other two treatments. These results suggest that there may have been higher blood insulin levels to stimulate greater glucose uptake (lower blood glucose), thereby triggering more rapid glycogen synthesis in the first hour of recovery.

D. Discussion:

During the fourth year of this project we have collected enough data to allow us to draw some conclusions about gender differences during our prolonged exercise protocol. We have studied the four basic hypotheses that were originally proposed and contracted, with minor revisions to hypothesis III. Our results and conclusions are discussed below:

Hypothesis I: ***There are differences in muscle recruitment patterns of men and women when an identical, non-normalized (i.e.-same mass, same duration) repetitive lifting task is performed over a prolonged period.*** The prolonged repetitive lifting task can be accomplished by both genders; however, a smaller percentage of women (33%) than men (100%) are able to complete all 12 blocks of exercise. The T2 increases in women are universally greater in women than in men (3, this report), indicating that women must work harder to accomplish the same task (Table 1a & 1b, Figure 1a & 1b, Appendix 1a & 1b). Women must also use a greater number of muscles to accomplish the task (Table 1a & 1b, Figure 1a & 1b, Appendix 1a & b) (3,this report). As the exercise protocol proceeds into the latter stages (blocks 8-12) men do not have to work any harder than they did at the start of

exercise (block 1); however, in the female population there is a trend toward increasingly harder work (Figure 2 & Figure 3). We conclude that both men and women distribute a prolonged repetitive lifting task over a large number of muscles (more in women than in men), so that the total workload is shared and no individual muscle is heavily recruited. Furthermore, we conclude that women work all of the muscle groups that we tested harder than do men, and as exercise extends over a prolonged period women may need to work even harder. However, both genders are capable of accomplishing the task, and size appears to be the most important controlling factor.

Hypothesis II: ***There are gender differences in systemic carbohydrate balance during the performance of this same prolonged repetitive lifting task and during recovery from the task. These differences are the result of women having to work harder to perform the same task. Female menstrual cycle phase may have an effect upon the results.***

We have measured muscle glycogen depletion in the liver, thighs and upper arms of men and women in the luteal & follicular phases of their menstrual cycles. While we find no significant differences between the three groups in glycogen depletion rates in the thighs (m.vastus lateralis & m.vastus intermedius), there appears to be a trend toward glycogen sparing in the female subjects during the luteal phase of their menstrual cycle (Figure 5a). These observations are suggestive of muscles that are not having to work hard to perform the specified work. This makes sense, given that the total workload is distributed amongst so many muscles (see results of Hypothesis I). Furthermore, when the workload is light women may mobilize fat stores more efficiently than men. This would account for the trend toward glycogen sparing in the mid-luteal females. Based upon unreported data from our lab (3), we would speculate that the thigh muscles of both genders are working at less than 12-15% of their maximum capacity to accomplish our lifting & carrying task. When we compared glycogen depletion rates during the first hour of exercise with rates during the subsequent two hours we found that in all three populations glycogen depletion slowed or even reversed (female mid-follicular) (Figure 6a). However, the variability

of the data during the last two hours of exercise indicates that we will not be able to detect a significant slowing of glycogen depletion during this project.

Data on the biceps suggest that, while the men only minimally work their left biceps muscles (less than their quadriceps), the trend in women is to deplete glycogen in their biceps muscles at a higher rate than their quadriceps muscles. Furthermore, women in both phases of their menstrual cycle deplete biceps glycogen at a significantly greater rate than do men (Figure 5b). A comparison with our previous data (3) suggests that we might speculate that women are working at around 20% of their maximum capacity. As we reported last year, these biceps glycogen depletion rates support the prediction of our MRI data that demonstrates greater recruitment of the left biceps in the female population. As with the quadriceps, male glycogen depletion rates were the same or slower during the last two hours of exercise than during the first hour (Figure 6b). However, during both phases of their menstrual cycle women seemed to deplete glycogen at a greater rate in the final two hours of exercise (Figure 6b). Again, the high degree of variability indicates that these observations will not become significant during this project, and thus will only be seen as trends. Taken as a whole, muscle glycogen measurements in both of the muscles that we studied indicate that glycogen is not severely depleted in either gender with our prolonged lift and carry protocol. Therefore, fatigue during this type of exercise is not likely to result from muscle glycogen depletion in either gender.

The net liver glycogen measurements obtained thus far reverse last years report of a difference between the male population and the female/luteal population. It now appears that there are no significant differences in net liver glycogen depletion in the three groups studied (Figure 7). As we discussed in our last yearly report, the liver measurements are complicated by the liver's ability to turnover its glycogen stores so that blood glucose concentrations can be maintained. Muscles do not have the enzyme necessary to cleave the terminal phosphate group from glucose-6-phosphate (glucose-6-phosphatase) and allow the release of glucose from muscle cells. The liver also has the ability to rapidly synthesize glycogen from three-carbon compounds generated by muscle metabolism and taken up by the liver (gluconeogenesis).

UNPUBLISHED DATA

Therefore, our measurements cannot track turnover and only indicate the net liver glycogen concentration at the time of measurement. However, by obtaining these net liver glycogen measurements we can get some sense of the systemic carbohydrate balance that is moderated by the liver. Our data indicate that the prolonged lift and carry exercise protocol does not significantly challenge the liver's ability to maintain readily available carbohydrate stores.

Hypothesis III: ***The administration of a carbohydrate supplement immediately before and after the performance of the same prolonged repetitive lifting task may have a glycogen sparing effect, and this effect may be different in men and women.***

We have measured muscle glycogen recovery in the thighs (m.vastus lateralis and m.vastus intermedius) of trained cyclists following an exhaustive bout of cycling exercise. We found that the exercise successfully depleted greater than 100mmoles of glycogen. A comparison under different nutritional conditions revealed that when a mixture of carbohydrate and protein is administered [~75% carbohydrate (80g) + ~25% protein (28g)] there is a trend toward an enhancement of glycogen recovery during the first hour following exercise (Figure 8a & 8b). However, preliminary data suggests that, by comparison, an iso-caloric amount of carbohydrate alone produces a glycogen recovery rate that is slower on average, but the rate is not likely to be significant during the course of this project. When the caloric value of the carbohydrate alone is reduced by ~25% glycogen recovery rates are significantly reduced (Figure 8b). We believe that the reason for the trend in protein enhancement of muscle glycogen recovery may be an increased insulin response to the addition of protein into the nutritional supplement. Plasma insulin samples have been obtained during this study, and we are currently waiting for the results of the analysis. We do have the results of glucose analysis that reveal significantly lower blood glucose levels during the first hour of recovery by subjects given the carbohydrate + protein supplement (Figure 9). The glucose results suggest that there may have been an enhanced insulin response in this group that increased the glucose clearance rate. As

a whole, our preliminary results point to a possible enhanced ergogenic effect of nutritional supplements that contain a protein component.

We would like to point out that these results are preliminary (n=3 in each group), and the conclusions are therefore not final. However, the trends appear to be solid, and we believe that they will hold true when full data sets are collected. We want to further point out that our study has not addressed the original hypothesis III as stated in the contracted proposal. We have altered the exercise protocol in order to focus the work on a specific set of muscles, and therefore produce severe glycogen depletion. We did this because the exercise protocol as originally described did not deplete enough glycogen to make this study relevant. Further, we believe that soldiers in the field will be faced with physical challenges that could be expected to produce this level of glycogen depletion. We have not administered a nutritional supplement prior to exercise because is not customary with the exercise protocol as now performed. We are currently studying only male subjects in this protocol, and expect that this study will be a male study. We have made this alteration because of the availability of the Yale University male intercollegiate cycling team.

Hypothesis IV: ***Four consecutive days performance of the same prolonged repetitive lifting task causes an overall downward trend in carbohydrate stores. This downward trend is the result of incomplete recovery from each previous day's exercise. The trend may be more pronounced in women than in men.***

We have tested only one additional subject in our study to examine the effect of performing our prolonged lift and carry exercise protocol on four consecutive days. Again, the preliminary data suggests a trend toward progressive supercompensation as the protocol continues over four days. This supercompensation occurs in the absence of a high carbohydrate diet, and suggests that the body can completely recover from the protocol overnight and prepare for the next day's demands. We do not yet have enough data to compare genders, although the data that we do have suggest similar patterns in the different genders.

E. Relationship to the Statement of Work (SOW) outlined in the proposal:

This contract was scheduled to expire in September of 2000 and has been extended in order to complete the research (no-cost extension to September 2001). While the administration of this grant continues to suffer from a difficulty in recruiting appropriate test subjects in the private sector, we have had some success in gaining access to the Yale University cycling team. When compared with both the original SOW and the revised SOW, we are still lagging behind in all areas of the project. Fortunately, all experimental procedures that have been undertaken have produced interesting results. We intend to continue accumulating studies beyond the term of the grant until we have reached definite conclusions in all phases of the project, and will request a second no-cost extension if needed. We would like to make the funding agency aware that we are currently in the process of upgrading the research facilities here in our laboratory, and we expect that the primary system upon which these studies are performed will go off line in March halting all experiments. A new system is expected to be operational in July, and experiments are expected to resume at that time. This process represents a loss of valuable time and an inconvenience; however, the new 4.0T high-field system will significantly improve the quality of data. The director of the MR center has assured me that, until this contract is completed, we will get time on the new system at the old rate. Unfortunately, it is likely that this upgrading process will necessitate a further no-cost extension. However, based on the results thus far we still believe that fewer studies will be required in order to complete the aims of the project. We have increased the subject reimbursement in order to attract a greater number of potential subjects and this seems to have helped the subject recruitment process to some extent.

F. Negative results:

Aside from our ongoing problems with subject recruitment and the interruption of experiments by our system upgrade, we have experienced no negative results in

accomplishing the experiments. We had one subject who complained of a persistence of soreness as a result of the study, and we reported the incident to the human investigation committee. When the subject complained we advised him that if he felt that his participation in the study had caused a problem he should report the problem. We further advised him that, as indicated in his signed subject consent form, he was entitled to any treatment deemed necessary as the result of problems associated with his participation in the study. He informed us at that time that he did not feel that his persistent soreness would be a problem.

G. Problems in accomplishing tasks:

The problems that were reported in our earlier annual reports (2,3) have persisted during the past year, and continue to slow the project. The problem of subject recruitment is particularly bothersome; however, the increase in subject pay has helped. We will continue to recruit as many subjects as we can with the intention of continuing our studies beyond the term of the contract.

KEY RESEARCH ACCOMPLISHMENTS

Year 1:

- Developed an echo-planar MRI protocol
- Designed and constructed a lift & carry exercise apparatus

Year 2:

- Demonstrated that MRI is capable of detecting gender differences in the lift & carry task
- Demonstrated that MRI can point to the muscles best studied with MRS

- Determined that liver glycogen is not significantly depleted by the lift & carry protocol in either gender
- Determined that quadriceps muscle glycogen is nominally depleted by the lift & carry protocol in both genders

Year 3:

- Demonstrated with MRI that women performing the lift & carry protocol recruit their upper body muscles, particularly arms, to a greater extent than do men
- Demonstrated with MRI that as the prolonged lift & carry protocol continues the whole body muscle recruitment pattern remains the same as following the initial exercise period
- Demonstrated with MRI that the quadriceps muscles are consistently recruited by the lift & carry protocol in both genders
- Demonstrated with MRI that the biceps muscles are recruited to a significantly greater extent by the lift & carry protocol in women
- Determined that the biceps would provide the greatest opportunity to demonstrate metabolic gender differences during the lift & carry protocol using MRS
- Demonstrated with MRS that, during lift & carry exercise, women in the luteal phase of their menstrual cycle deplete liver glycogen at a significantly slower rate than do men.
- Determined that with the current variability gender differences in biceps glycogen depletion rates will be demonstrated in this project

Year 4:

- Demonstrated with MRI that in women, as prolonged exercise continues into the third hour, the change in T_2 increases in the lower legs indicating that they are working harder

- Developed a volume filling process the allows multi-slice 2-D transverse MR images to be rendered in 3-D, thereby allowing T_2 data to be dumped into reconstructed male and female images of subjects from the study
- Demonstrated with MRS that during the prolonged lift & carry task women deplete glycogen from their biceps muscles at a significantly greater rate than do men, thereby confirming the prediction of the MRI study
- Reversed the previous demonstration of menstrual cycle variations (luteal phase) in liver glycogen depletion rates
- Demonstrated with MRS and blood analysis that adding protein to post-exercise nutritional supplements may provide a benefit

REPORTABLE OUTCOMES

2000: (September 20-23, Portland, ME) American Physiological Society Intersociety Meeting, The Integrative Biology of Exercise, [ABSTRACT entitled: "MRI of Gender Differences in Exercise" APS Quarterly 43(4), p.362.

2000: (September) National Geographic, Section entitled "The Inner Workings of Fitness", pp. 14-15, in an article entitled "What it Takes to Build the Unbeatable Body: Pushing the Limits" by Rick Gore. This section uses the 3-D volume filling technique that will be used to present our MRI data in publication.

CONCLUSIONS

From this fourth year of our project we conclude that, while the male muscle recruitment pattern does not change as the exercise session continues, women recruit their lower legs to a greater extent in the third hour of exercise. Therefore, although new muscles are not recruited to compensate for fatiguing muscles, in women some of the muscle that are recruited have to work harder in the latter stages of the prolonged lift and carry exercise employed in this study. Biceps glycogen depletion rates during the lift and carry protocol are significantly faster in women than in men.

This finding represents the first time that MRS has been used to confirm MRI as a means of predicting muscle glycogen metabolism. Finally, we predict that when more studies are completed we will see a beneficial effect of adding protein to post-exercise nutritional supplementation.

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8. Fisher, MJ, et al. *Invest. Radiol.* 25: 480-485, 1991.

UNPUBLISHED DATA (all appendices, tables, and figures)

LEGEND

Appendices:

1a. Schedule of APS meeting indicating the session of our abstract

1b. Copy of the abstract from the APS Quarterly, Vol. 43 No. 4, p.362.

Tables:

1a. The change in T_2 for 20 muscles of the upper body following a single 15min bout of lift & carry exercise in the male and female populations. If the muscle is labeled as not activated, it means that the T_2 change was tested and found to be not significant. The level of significance of individual muscle T_2 change between men and women is given in column 4.

1b. The change in T_2 for 20 muscles of the lower body following a single 15min bout of lift & carry exercise in the male and female populations. If the muscle is labeled as not activated, it means that the T_2 change was tested and found to be not significant. The level of significance of individual muscle T_2 change between men and women is given in column 4.

<u>Figures:</u>

1. a. Map of T_2 change [msec] in the male population calculated from all muscles that were significantly recruited. b. Map of T_2 change [msec] in the female population calculated from all muscles that were significantly recruited.
2. Average T_2 change [msec] calculated from the: a. arms & shoulders, b. trunk, and c. gluteal muscles during three hours of lift & carry exercise. Data are from male (n=6) and female (n=6) subject populations. * $p \leq 0.05$ men versus women.
3. Average T_2 change [msec] calculated from the: a. quadriceps, b. hamstrings, and c. lower leg muscles during three hours of lift & carry exercise. Data are from male (n=6) and female (n=6) subject populations. * $p \leq 0.05$ men versus women. $p=0.0403$ men first versus second hour, $p=0.0239$ women first versus third hour.
4. This figure is an example of the recently developed 3-D volume filling process. The figure represents lower leg recruitment, measured as T_2 change, by plantar flexion of the foot at three different knee orientations (0° flexion, 45° flexion, and 90° flexion). The figures are 3-D representations of 2-D data (see insets). This figure is not intended to present data, rather it is included in this report as a demonstration of the was in which we intend to present total body data in the publication of the MRI portion of the project.
5. a. Quadriceps glycogen depletion rates [mmol/l-hr] in male & female populations during three hours of lift & carry exercise. b. Biceps glycogen depletion rates [mmol/l-hr] in the same populations during the same exercise period ($p=0.0050$ male versus female mid-follicular, $p=0.0184$ male versus female mid-follicular). Data are mean±SE.

6. a. Quadriceps glycogen depletion rates [mmol/l-hr] in male & female populations during 0-1hours and during 1-3 hours of lift & carry exercise. b. Biceps glycogen depletion rates [mmol/l-hr] in the same populations during the exercise periods Data are mean±SE.
7. Liver glycogen depletion rates during the lift & carry protocol in male and female populations. Data are mean±SE.
8. a. Time-course of quadriceps glycogen [mmol/l] recovery during 4 hours following a 2 hour bout of bicycling at 70% of VO_{2MAX} + a series of sprints at 100% of VO_{2MAX}. On different occasions subjects were administered one of three different post-exercise (immediately after exercise and 2hr after exercise) nutritional supplements. b. Quadriceps glycogen re-synthesis rates [mmol/l-hr] with the different nutritional supplements during 0-60min of recovery and during 60-240min. The nutritional supplements are: A. (80g carbohydrate, 28g protein, 9g fat), B. (108g carbohydrate, 9g fat), and C. (80g carbohydrate, 9g fat). *$p \leq 0.03$ versus supplement A. Data are mean±SE.
9. Blood glucose concentrations [mg/dl] at rest, during exercise, and during 4 hours of recovery for the different nutritional supplements. *$p \leq 0.04$ versus supplement A. Data are mean±SE.

APPENDIX 1a:

2000 APS Intersociety Meeting: THE INTEGRATIVE BIOLOGY OF EXERCISE

September 20-23, Holiday Inn By the Bay, Portland, Maine

Wednesday, September 20—Registration opens: 2:00 PM

Welcome and Opening Reception: 7:00-9:00 PM

THURSDAY SEPTEMBER 21	FRIDAY SEPTEMBER 22	SATURDAY SEPTEMBER 23
8:00-11:00 AM—Symposium **1.0 EXERCISE-INDUCED CARDIOPROTECTION: CELLULAR ASPECTS** E.G. Lakatta, Natl Inst Aging M Boluyt, U Michigan D. Korzick, Penn State U D. Bowles, U Missouri C. Bloor, UCSD	8:00-11:00 AM—Symposium **12.0 HOW DOES SKELETAL MUSCLE ADAPT TO EXERCISE?** B. Russell, U Illinois, Chicago M.T. Hamilton, U Texas Hlth Sci Ctr Houston S.J. Swoap, Williams Col K. Esser, U Illinois, Chicago C. Peterson, U Arkansas	8:00-11:00 AM—Symposium **23.0 THE ROLE OF PHYSICAL ACTIVITY IN THE PREVENTION OF OBESITY AND MANAGEMENT OF BODY WEIGHT** C. Bouchard, Pennington Biomed Res Ctr D. Schoeller, U Wisconsin D. York, Pennington Biomed Res Ctr M. Goran, U Alabama, Birmingham' T. Rankinen, Laval U E. Ravussin, Pennington Biomed Res Ctr O. Boss, Beth Israel Deaconess Med Ctr
8:00-11:00 AM—Symposium **2.0 EXERCISE AND AGING: CHALLENGE, RESILIENCY AND FUNCTION** K. Kregel, U Iowa L. DiPietro, Yale U R. Fielding, Boston U W. Kohrt, U Colorado HSC, Denver J.A. Taylor, Beth Israel Deaconess, Harvard U G.D. Cartee, U Wisconsin, Madison	8:00-11:00 AM—Symposium **13.0 GENDER-DEPENDENT RESPONSES TO EXERCISE** G. Brooks, UC Berkeley S.N. Davis, Vanderbilt U A.L. Friedlander, Stanford U A.B. Loucks, Ohio Univ S. Hopkins, UCSD R. Marcus, Standord U M.H. Laughlin, U Missouri	8:00-11:00 AM—Symposium **24.0 IMPACT OF TRANSGENIC MANIPULATIONS ON INTEGRATED EXERCISE PERFORMANCE** H.L. Sweeney, U Pennsylvania J. Robbins, Children's Hosp, Cincinnati L.A. Leinwand, U Colorado E.G. Kranias, U Cincinnati B. Roman, U Illinois Med Ctr E. Barton-Davis, U Pennsylvania
11:00 AM-2:00 PM **POSTER VIEWING AND DEFENDING:** **3.0 DEVELOPMENT AND AGING** **4.0 DIET, BODY COMPOSITION AND ENERGETICS** **5.0 ENDOCRINE RESPONSES TO EXERCISE** **6.0 INTEGRATED SYSTEMS** **7.0 METABOLISM—CARBOHYDRATE, FAT, PROTEIN, LACTATE**	11:00 AM-2:00 PM **POSTER VIEWING AND DEFENDING:** **14.0 AUTONOMIC NERVOUS SYSTEM** **15.0 CELLULAR REGULATORY MECHANISMS** **16.0 HEART AND CIRCULATION** **17.0 MOLECULAR REGULATORY MECHANISMS** **18.0 VASCULAR BIOLOGY**	11:00 AM-2:00 PM **POSTER VIEWING AND DEFENDING** **25.0 COMPARATIVE PHYSIOLOGY OF EXERCISE** **26.0 FATIGUE** **27.0 LUNGS, BLOOD, AND O_2 TRANSPORT** **28.0 MOTOR CONTROL, LOCOMOTION AND BIOMECHANICS** **29.0 MUSCLE DAMAGE AND DISEASE STATES**
2:00-3:00 PM—Tutorial **8.0 TO BE ANNOUNCED** 2:00-3:00 PM—Tutorial **9.0 MUSCLE GLYCOGEN METABOLISM: OLD CONCEPTS REVISITED** T. Graham, U Guelph	2:00-3:00 PM—Tutorial **19.0 NITRIC OXIDE AND EXERCISE: FUNDAMENTALS OF NITRIC OXIDE AND FREE RADICAL CHEMISTRY** G. Buettner, U Iowa	2:00-4:00 PM—Roundtable **30.0 THE GENES TO HEALTH INITIATIVE: A BOLD CHALLENGE TO THE PHYSIOLOGICAL AND EXERCISE SCIENCES** K. Baldwin, U California, Irvine F. Booth, U Texas Hlth Sci Ctr, Houston R. Armstrong, Texas A&M U M.H. Laughlin, U Missouri R. Lymn, NIAMSD, NIH E.E. Sinnett, NIH M. Frank, APS
3:00-4:00 PM—Tutorial **10.0 EXERCISE: WOMEN AT ALTITUDE** L. Moore, U Colorado, Denver B. Braun, U Massachusetts	2:00-300 PM—Tutorial **20.0 EXERCISE IN MICROGRAVITY** S. Schneider, NASA Johnson Space Ctr	
3:00-4:00 PM—Tutorial **11.0 MECHANISMS OF BODY HEAT ADAPTATION** M. Sawka, US Army Res Inst Environ Med	3:00-4:00 PM—Tutorial **21.0 CONTROL OF THE MUSCLE CIRCULATION** S. Segal, John B Pierce Lab and Yale U	4:00-6:00 PM **POSTER VIEWING AND DEFENDING**
4:30 PM—Party Boats depart for Peaks Island 5:30-9:30 **LOBSTER BAKE ON PEAKS ISLAND** (ticket purchase required)	3:00-4:00 PM—Tutorial **22.0 COMPARATIVE MODELS IN EXERCISE PHYSIOLOGY: ACCESSIBLE SYSTEMS TO ANSWER DIFFICULT QUESTIONS** J. Hicks, U California, Irvine 4:00-6:00 PM **POSTER VIEWING AND DEFENDING**	7:30-10:00 PM—Party **31.0 BANQUET AND AWARDS PRESENTATION** Featuring Guest Speaker: JAMES A. PAWELCZYK, Astronaut, Penn State U

Conference 2

27.19

THE DEPENDENCE OF ACTIVATED SWEAT GLANDS ON CHANGING SWEATING RATE DURING SUSTAINED STATIC EXERCISE IN HUMANS

N. Kondo, S. Yanagimoto, K. Aoki, M. Shibasaki* and Y. Inoue**
Fac. of Human Dev., Kobe Univ., Kobe 657-8501, *Nara Women's Univ., **Osaka International Women's Univ., Osaka, Japan.

To investigate how changes in the sweating rate (SR) with increasing exercise intensity during isometric handgrip exercise depend on activated sweat glands (ASG) and sweat output per gland (SGO), fourteen male subjects performed 20, 35, and 50% maximal voluntary contraction for 60 sec with the right hand. The experiment was conducted at an ambient temperature of 35°C and a relative humidity of 50%. In this environment, sudomotor was activated by the increased skin temperature, although there was no marked change in internal temperature. Heart rate, perceived rate of exertion and mean arterial pressure increased with exercise intensity ($p<0.05$). Although the body temperature (sublingual, local skin, and mean skin temperatures) remained essentially constant throughout the exercise at each intensity, the SR on the left forearm significantly increased with a rise in exercise intensity ($p<0.05$). This indicates that nonthermal factors contribute to the increase in the SR with intensity. At the site where the SR was measured, the change in ASG with rising exercise intensity was similar to that in the SR, while SGO did not change markedly with intensity. These results suggest that the increase in the SR with exercise intensity during sustained static exercise depends on ASG, but not on SGO. The effect of nonthermal factors on the change in the SR might influence the recruitment of activated sweat glands.

27.20

COLD ACCLIMATION AND ACUTE HYPOTHERMIA DIFFERENTIALLY AFFECT MUSCLE PERFORMANCE IN RATS AND HAMSTERS

D. Deveci & S. Egginton*. Dept. of Physiology, Medical School, University of Cumhuriyet, 58140 Sivas, TURKIYE and *University of Birmingham, UK.

Blood flow (BF) and muscle performance may be affected differently in non-hibernators (rat) and hibernators (hamster) by 8 weeks cold acclimation (CAc, Tamb=20-5oC) and hypothermia compared to controls. BF was measured by microspheres under anaesthesia during either normothermia (N) (Tcore=37oC) or hypothermia (H) (Tcore=25oC). Capillary/fibre ratio (C:F) was estimated in cryostat sections (alkaline phosphatase) and correlated with enzyme activity, arterial oxygen tension (PaO2), and heamatocrit (%Hct). Muscle performance was estimated by fatigue resistance index (FRI=final/peak tension x100) during 5 min isometric contractions of tibialis anterior (TA) and extensor digitorum longus (EDL). CAc significantly increased C:F in rat TA (1.34±0.08 to 1.72±0.08), P<0.05) but not in hamtser TA (2.13±0.07). Citrate synthase and 3-hydroxyacyl-CoA dehydrogenase were unaltered by CAc, although they were ~30% and ~65%, higher in hamster, respectively. Hct% was increased from 39 to 43% in rat by CAc while it was maintained in hamster (48%). PaO2 was similar in between control and CA rats during N and H (~90%), while in hamster PaO2 was similar under N (~60%) but it was significantly lower under H in both groups (~30%). BF was higher in hamster (~155 ml/min/100g) than rat (~105) in all conditions, but there were no significant alterations on CAc. In rat, FRI% was 63% in both control and CA groups while under H it reduced by 39% and 48%, respectively. In hamsters, there was no significant alterations in FRI% under any condition (~90%). In conclusion, CAc of rat improved muscle performance under H which may be due to cold-induced angiogenesis, Hct%, or thermogenic capacity. In hamsters, higher FRI% may be due to high oxidative capacity, capillarity, and/or inherently elevated tolerance capacities to H.

MOTOR CONTROL, LOCOMOTION AND BIOMECHANICS

28.1

EFFECTS OF WALK TRAINING ON GLIAL CELL LINE DERIVED NEUROTROPHIC FACTOR (GDNF) EXPRESSION IN SKELETAL MUSCLE.

E.A. Wehrwein, E.M. Roskelley, and J.M. Spitsbergen, Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008

Glial cell line-derived neurotrophic factor (GDNF) is a recently discovered neurotrophic factor that affects peripheral motor neurons. Increased expression of GDNF, in skeletal muscle, leads to an increase in axonal branching and synapse formation. Studies examining exercise-induced changes in neuromuscular structure and function have demonstrated that increased physiological activity causes significant changes in neuromuscular junction complexity in the *extensor digitorum longus* and *soleus*. The goal of the present study was to determine whether altered neuromuscular activity leads to changes in GDNF expression in skeletal muscle. The hypothesis being tested states that increased neuromuscular activity will cause an increase in skeletal muscle GDNF expression. Adult, male Sprague-Dawley rats were used in this study. Following 4 week of walk training on a treadmill, skeletal muscle samples were removed and analyzed for GDNF content. The data represent mean levels of GDNF (pg GDNF/mg tissue wet weight). Gastrocnemius muscle from sedentary control rats contained significantly lower levels ($p \leq 0.05$) of GDNF (35.3 pg/mg) than gastrocnemius muscle from exercise rats (260 pg/mg). GDNF levels in the vastus medialis muscle from control rats (8.4 pg/mg) was also significantly lower ($p \leq 0.05$) than levels from exercise rats (239.6 pg/mg). The results demonstrate that increased levels of physical activity do alter GDNF expression in skeletal muscle. The altered production of GDNF may be responsible for the changes in complexity of the neuromuscular junction seen with increased physiological activity. Supported by NIH grant R15 HL60240-01 and the Faculty Research and Support Fund, Western Michigan University

28.2

ABSTRACT WITHDRAWN.

28.3

ENERGETICS OF HIGH-SPEED RUNNING PERFORMANCE

P.G. Weyand, M.W. Bundle, and S. Lee; CFS, Harvard University, Bedford, MA 01730; USARIEM, Natick, MA 01760

We hypothesized that maximal speed during all-out running can be predicted from the peak speeds supported by aerobic and anaerobic power. We determined peak aerobic speeds from measurements of aerobic power and the metabolic cost of steady-state running, and peak anaerobic speeds from the highest speed attained during progressive treadmill efforts of ~ 5 s. Five sprinters (S), 5 middle-distance runners (MD), and 7 long distance runners (LD) completed progressive discontinuous treadmill tests (4.6°) and 11-15 all-out runs at speeds that elicited exhaustion in 10 to 180 s. Top burst speeds were greatest for S (8.9 ± 0.2 m/s), intermediate for MD (8.5 ± 0.5 m/s) and slowest for LD (6.7 ± 0.6 m/s), while top aerobic speeds were slowest for S (3.2 ± 0.2 m/s), and equivalent for LD (4.0 ± 0.4 m/s) and MD (4.0 ± 0.3 m/s). We predicted the speeds measured during all-out runs that spanned a 12-fold range of run durations, and a 2-fold range of absolute speeds (n = 247 runs, $R^2 = 0.975$) from top burst speed, top aerobic speed, and the time of the run. We conclude that the relationship between mechanical performance and the chemical energy provided by aerobic and anaerobic sources does not vary among different runners.

28.4

MRI of Gender Differences in Exercise. **T.B. Price & K.M. Johnson**

This study used magnetic resonance imaging (MRI) to compare muscle recruitment patterns in male and female subjects performing a lift and carry exercise protocol. Six males (25±3yrs, 85±3kg, 180±3cm) and six females (27±3yrs, 60±3kg*, 165±3cm*, $p \leq 0.005$) repeatedly lifted a 30kg box from floor level, carried it 3m, and placed it at a height of 132cm. Subjects performed 3 lifts/min over a 15min period. T2-weighted transverse MR images (180 slices) were obtained from the whole body before and immediately after exercise. Fifty-two muscles were assessed for exercise induced T2 increase. Gender differences were noted in the total number of muscles with significantly increased T2 ($p \leq 0.05$), males recruited 19±2 different muscles and females recruited 34±1 ($p \leq 0.002$). Females increased T2 times in the upper body more than males (4.0±0.5msec, F; 1.8±0.4msec, M; $p \leq 0.005$). Both genders produced similar T2 increases in the lower body (3.9±0.3msec, F; 3.3±0.3msec, M). The biceps exhibited the most significant difference between genders (5.7±0.7msec, F; 2.3±0.5msec, M; p=0.0013). Triceps and chest muscles were not significantly recruited in either sex. We conclude that MRI can be used to detect gender differences following an exercise task that requires a complex series of movements. We further conclude that, while both genders use their lower bodies to perform the lift and carry task, females use their upper bodies to a greater extent than do males.

Funding Source: U.S. Army DAMD17-96-C-6097

Conference 2

Table 1a:

UPPER BODY

Muscle	FEMALES ΔT_2 [msec]	MALES ΔT_2 [msec]	m vs f significance
L.deltoid	3.2±1.7	not activated	-----------
R.deltoid	3.2±0.7	not activated	-----------
L.bicep	5.8±0.9	2.5±0.4	p=0.0073
R.bicep	6.1±1.1	2.1±0.5	p=0.0079
L.posterior f.a.	6.1±0.7	2.8±1.1	p=0.0275
R.posterior f.a.	5.4±1.4	3.3±1.2	-----------
L.anterior f.a.	4.0±0.9	3.9±0.6	-----------
R.anterior f.a.	5.5±0.4	4.2±0.6	-----------
L.trapezius	2.9±0.6	not activated	p=0.0178
R.trapezius	4.0±0.9	not activated	p=0.0023
L.latissimus d.	3.0±0.6	not activated	p=0.0181
R.Latissimus d.	2.4±0.4	not activated	p=0.0334
L.lower back	4.1±1.2	5.0±1.1	-----------
R.lower back	4.6±1.1	4.4±0.5	-----------
L.&R. triceps	not activated	not activated	-----------
L.&R. p.major	not activated	not activated	-----------
L.&.R. p.minor	not activated	not activated	-----------

Table 1b:

LOWER BODY

Muscle	FEMALES ΔT2 [msec]	MALES ΔT2 [msec]	m vs f significance
L.r.femoris	4.0±0.5	1.9±0.6	p=0.0036
R.r.femoris	3.7±0.6	2.0±0.5	p=0.0334
L.v.lateralis	4.5±0.6	3.2±0.3	p=0.0376
R.v.lateralis	4.6±0.8	3.6±0.5	-----------
L.v.intermedius	4.4±0.5	2.8±0.6	p=0.0222
R.v.intermedius	4.3±0.7	3.5±0.3	-----------
L.v.medialis	4.6±0.8	3.0±0.7	-----------
R.v.medialis	4.7±0.9	3.5±0.5	-----------
L.sartotius	2.4±0.5	2.4±0.7	-----------
R.sartorius	4.8±1.0	2.6±0.7	-----------
L.b.femoris	5.9±0.7	4.4±0.4	-----------
R.b.femoris	6.0±0.8	4.9±0.5	-----------
L.semimembranosus	5.2±0.8	3.3±0.6	-----------
R.semimembranosus	5.9±1.2	3.7±0.9	-----------
L.semitendinosus	6.0±1.1	3.9±0.3	p=0.0466
R.semitendinosus	3.7±1.0	3.6±0.7	-----------
L.gastrocnemius	3.1±1.0	2.8±0.9	-----------
R.gastrocnemius	3.8±1.0	2.8±0.7	-----------
L.soleus	2.8±1.2	2.2±1.1	-----------
R.soleus	2.9±1.0	not activated	-----------
L.t.anterior	2.5±0.9	not activated	-----------
R.t.anterior	2.6±1.1	2.6±1.2	-----------
L.g.maximus	5.0±1.6	3.1±0.6	-----------
R.g.maximus	4.5±1.0	2.1±0.9	p=0.0460
L.g.medius	5.1±1.5	2.9±0.4	-----------
R.g.medius	5.0±1.7	2.5±0.5	-----------
L.g.minimus	2.4±0.7	2.1±1.1	-----------
R.g.minimus	3.8±1.2	2.3±1.0	-----------

Figure 1a:

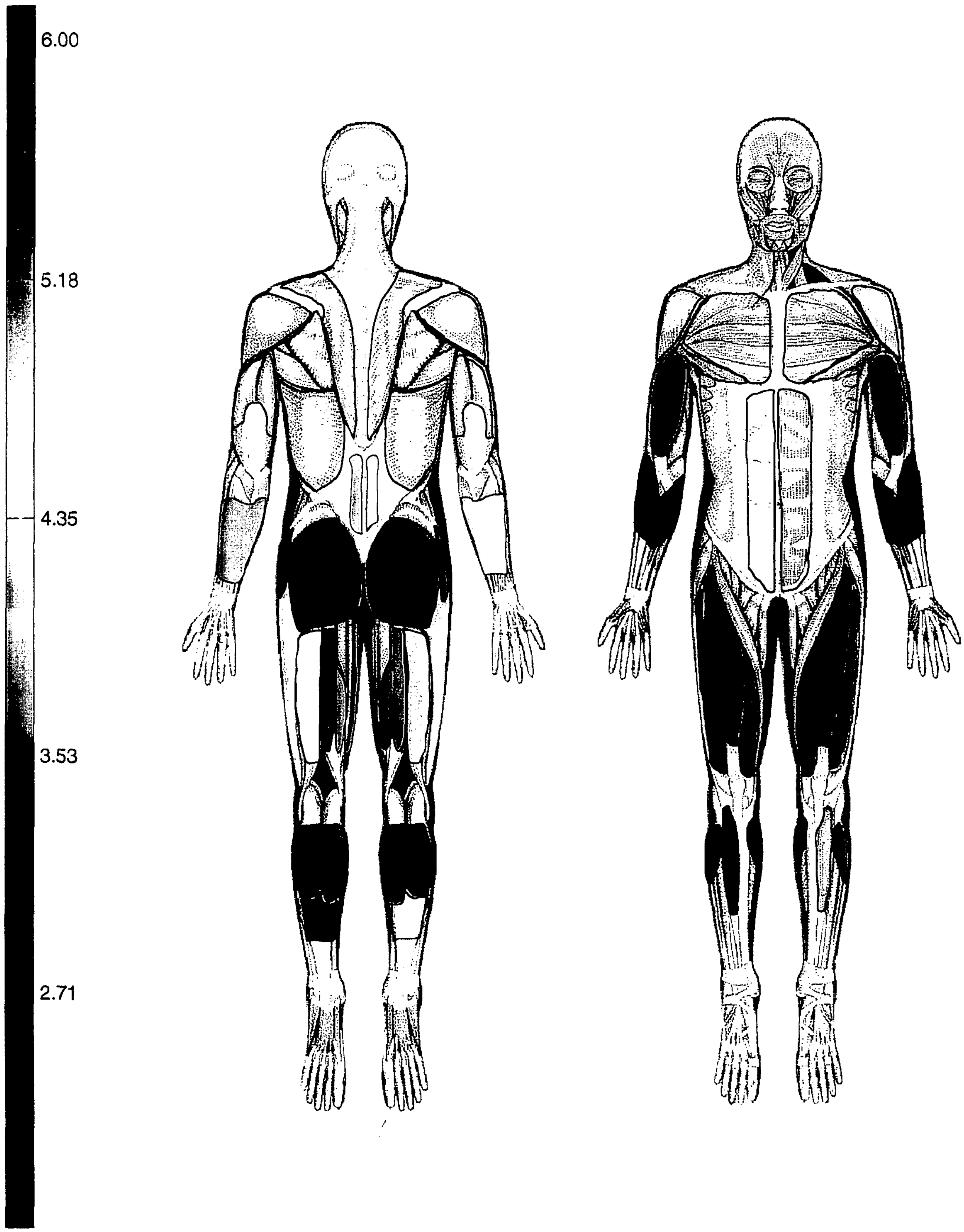


Figure 1b:

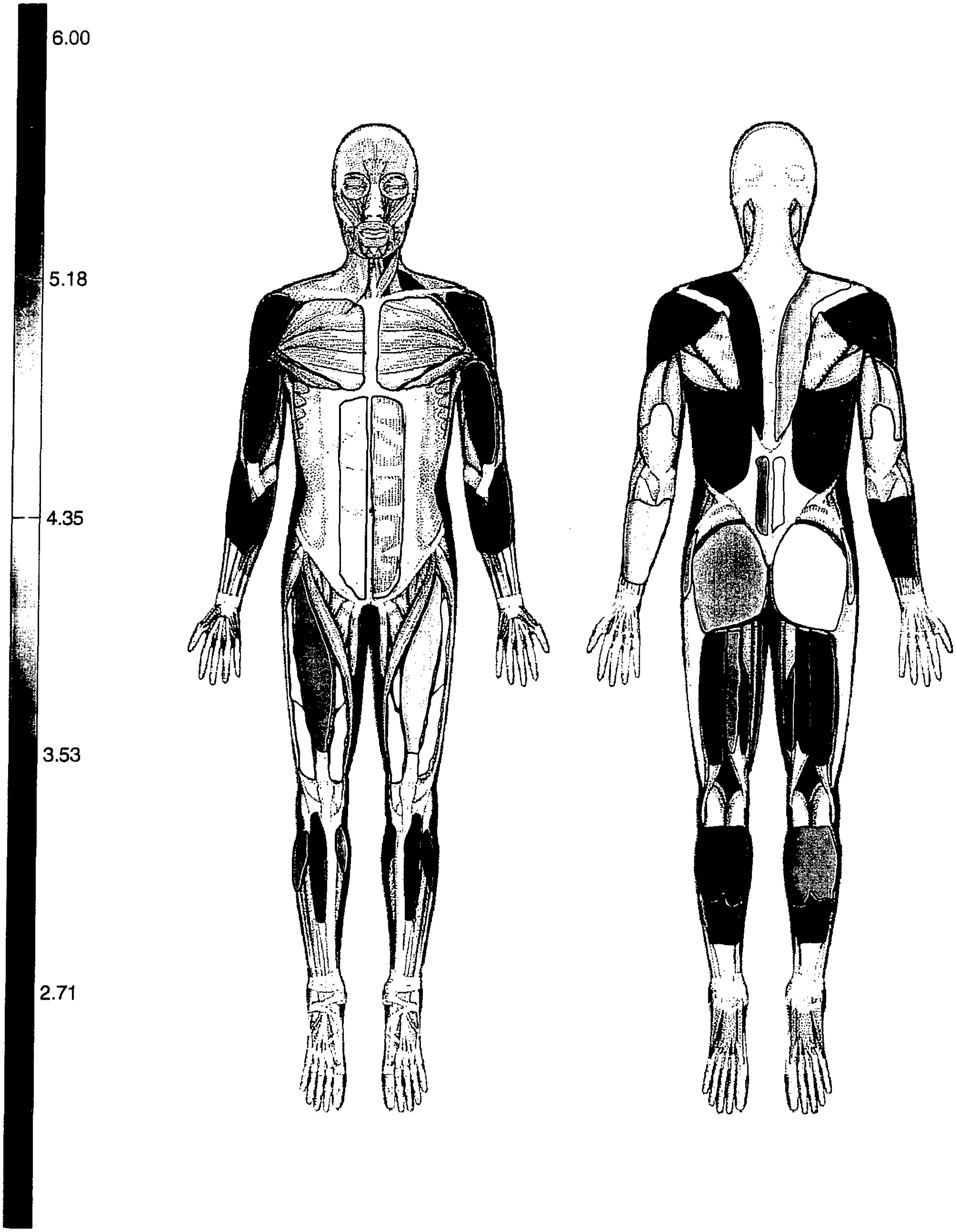
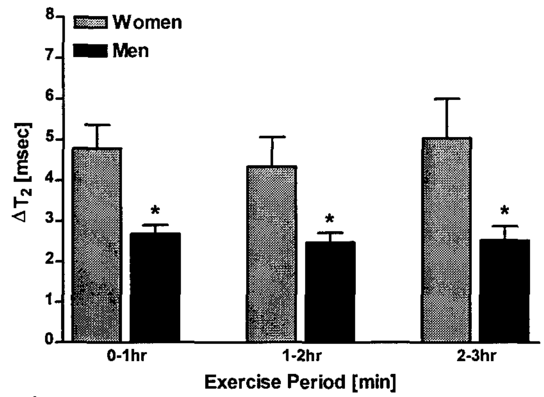


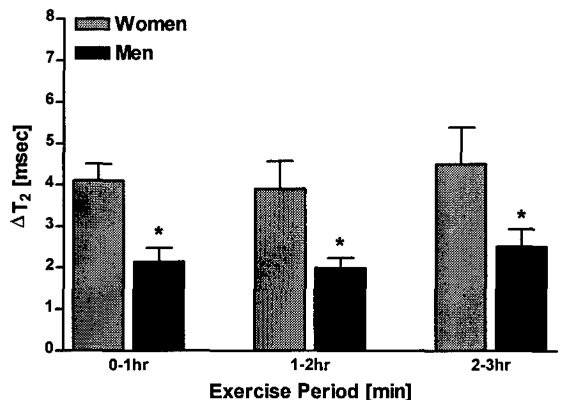
Figure 2:

a. ARMS & SHOULDERS



* p≤0.04 m vs f

b. TRUNK



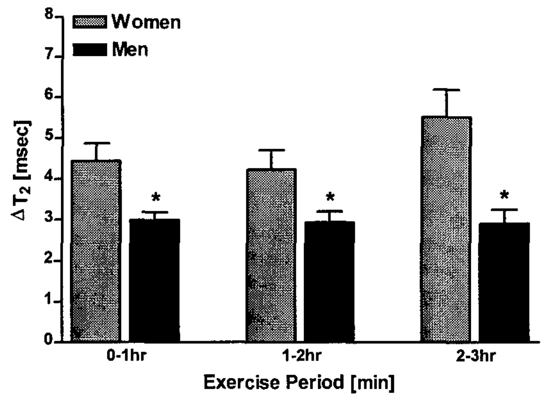
* p≤0.04 m vs f

c. GLUTEALS

* p≤0.02 m vs f

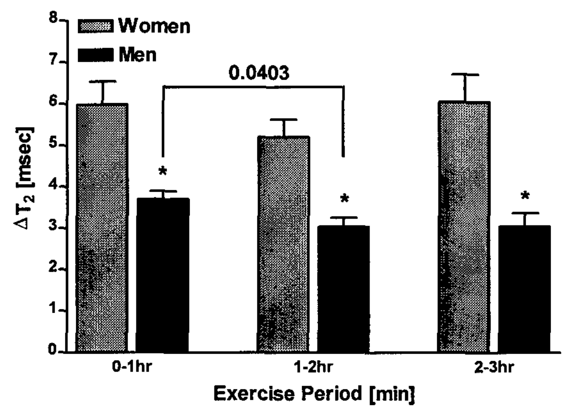
Figure 3:

a. QUADRICEPS



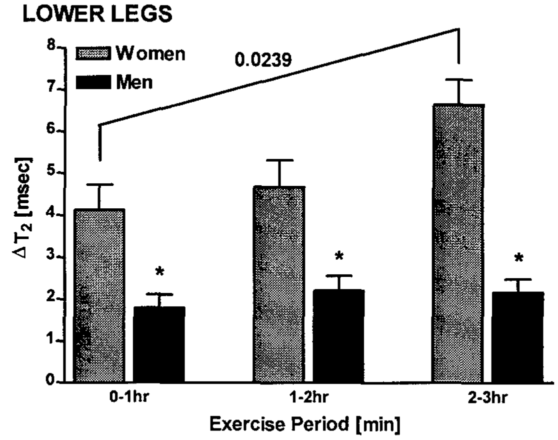
* p≤0.04 m vs f

b. HAMSTRINGS



* p≤0.0004 m vs f

c. LOWER LEGS



* p≤0.002 m vs f

Figure 4:

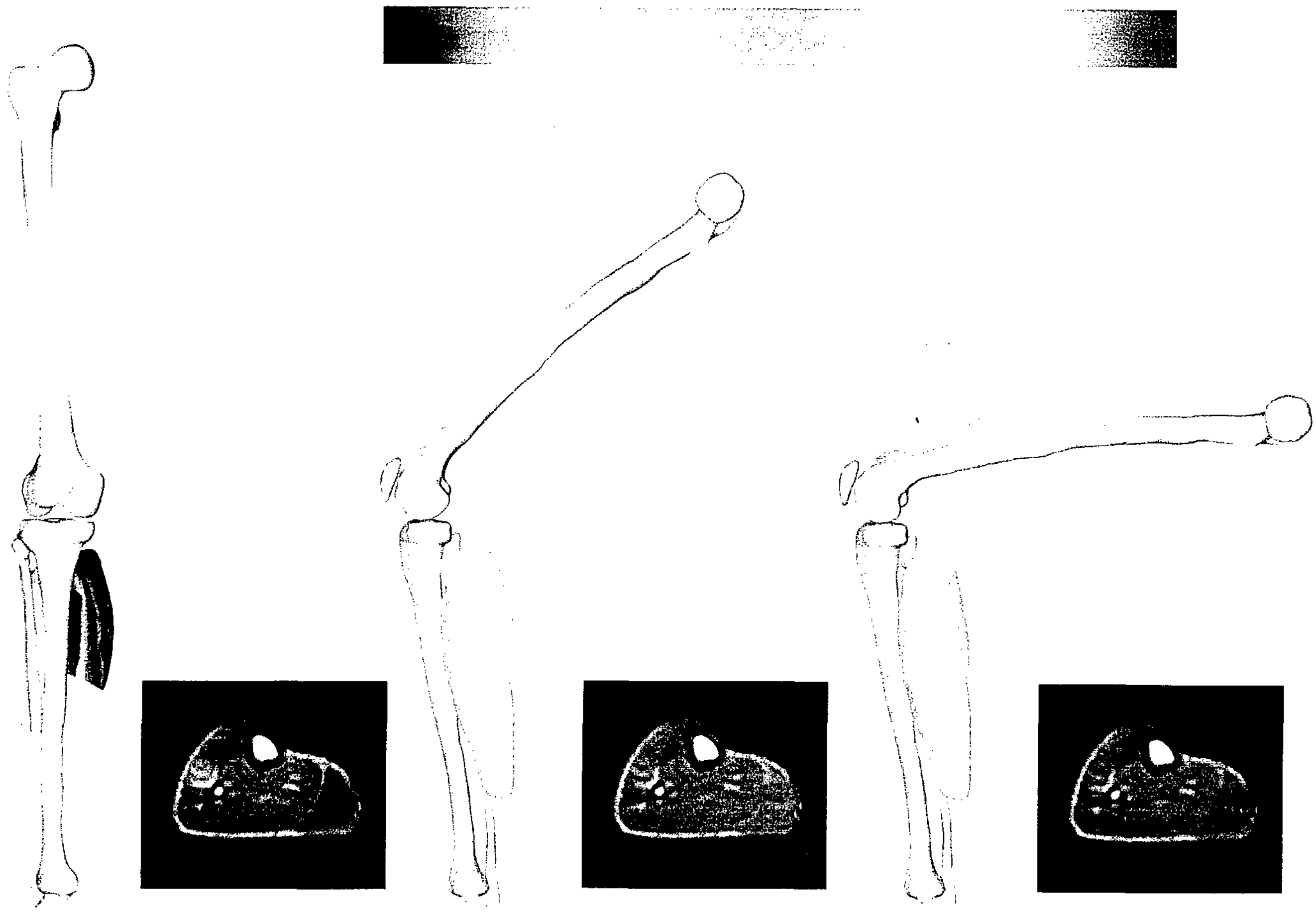
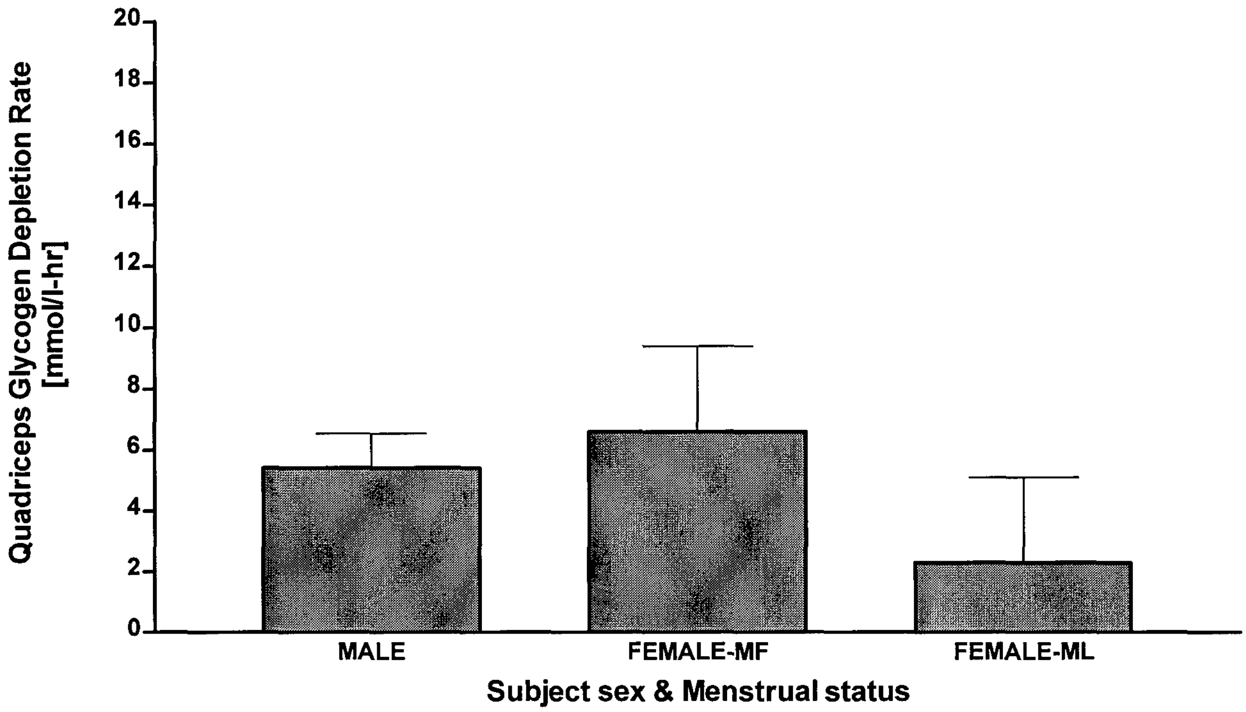


Figure 5a:


Quadriceps Glycogen Depletion Rate
[mmol/l-hr]
20
18
16
14
12
10
8
6
4
2
0
MALE
FEMALE-MF
FEMALE-ML
Subject sex & Menstrual status

5b:

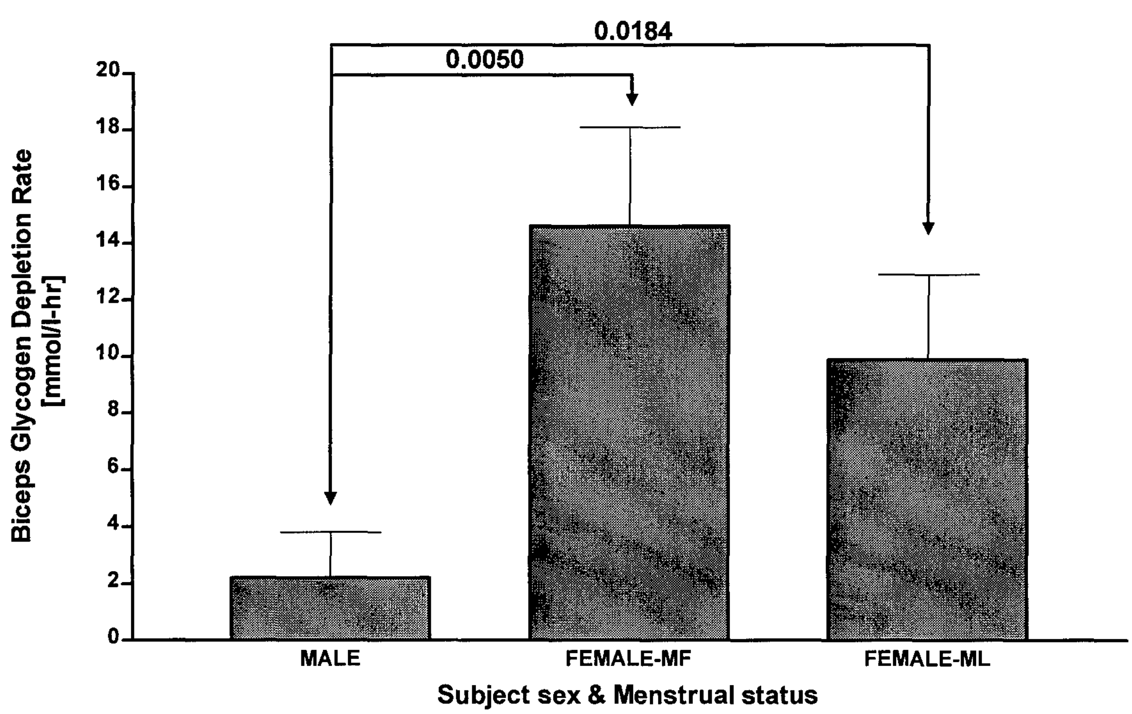
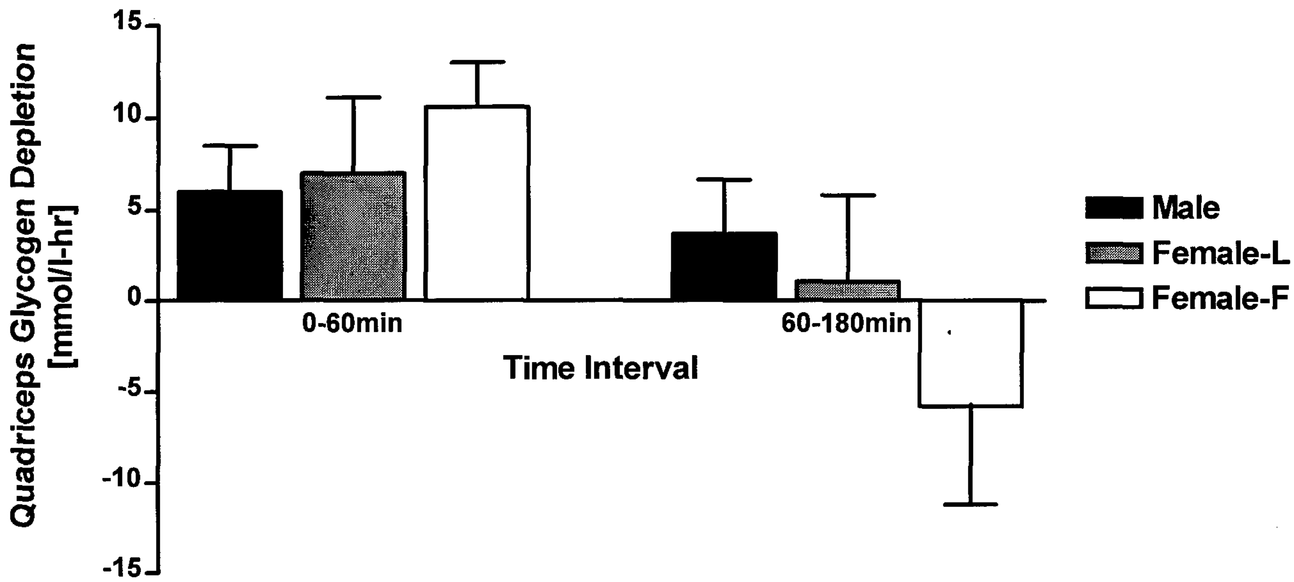

0.0184
0.0050
Biceps Glycogen Depletion Rate
[mmol/l-hr]
20
18
16
14
12
10
8
6
4
2
0
MALE
FEMALE-MF
FEMALE-ML
Subject sex & Menstrual status

Figure 6a:


Quadriceps Glycogen Depletion [mmol/l-hr]
15
10
5
0
-5
-10
-15
0-60min
60-180min
Time Interval
Male
Female-L
Female-F

6b:

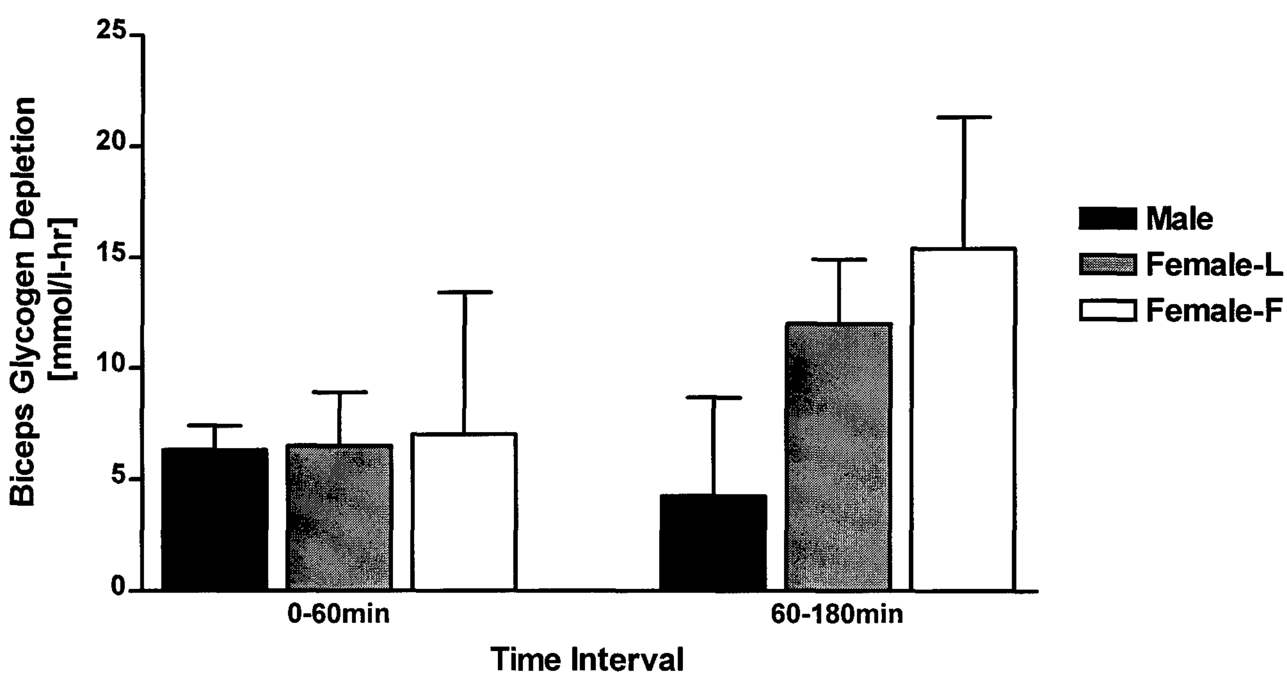

Biceps Glycogen Depletion [mmol/l-hr]
25
20
15
10
5
0
0-60min
60-180min
Time Interval
Male
Female-L
Female-F

Figure 7:

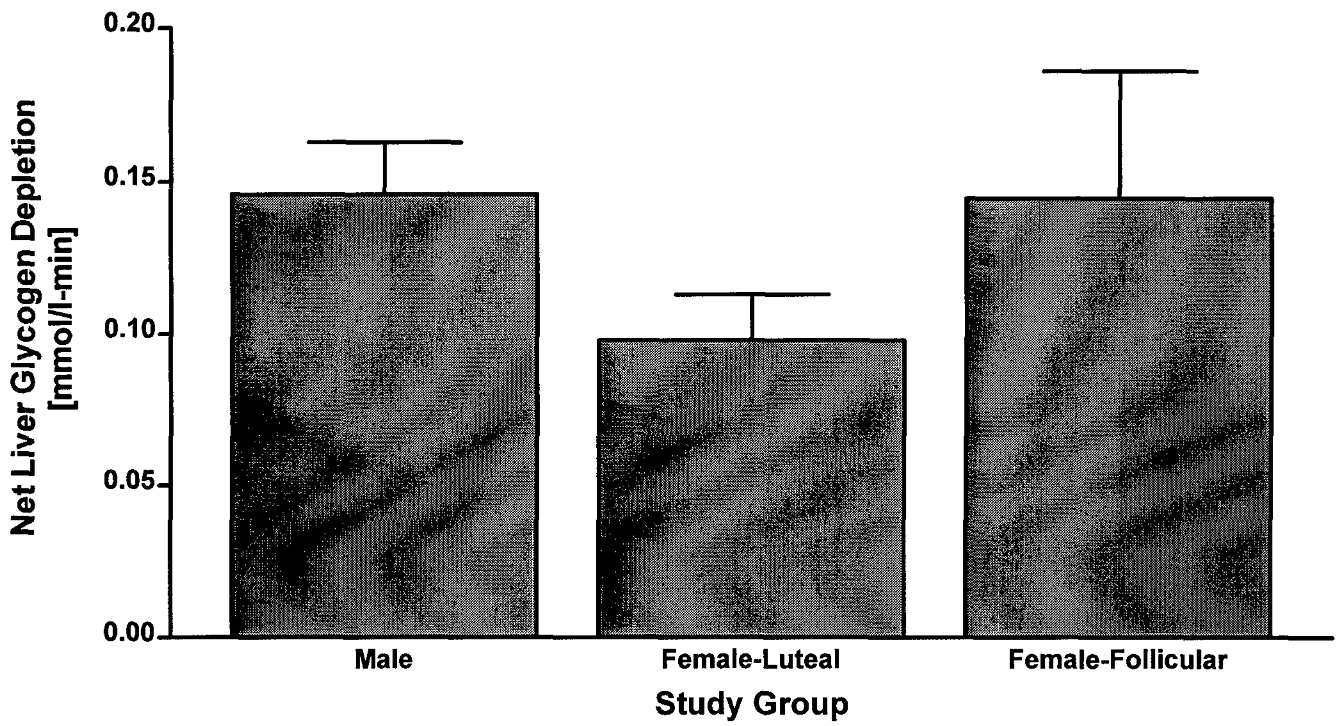
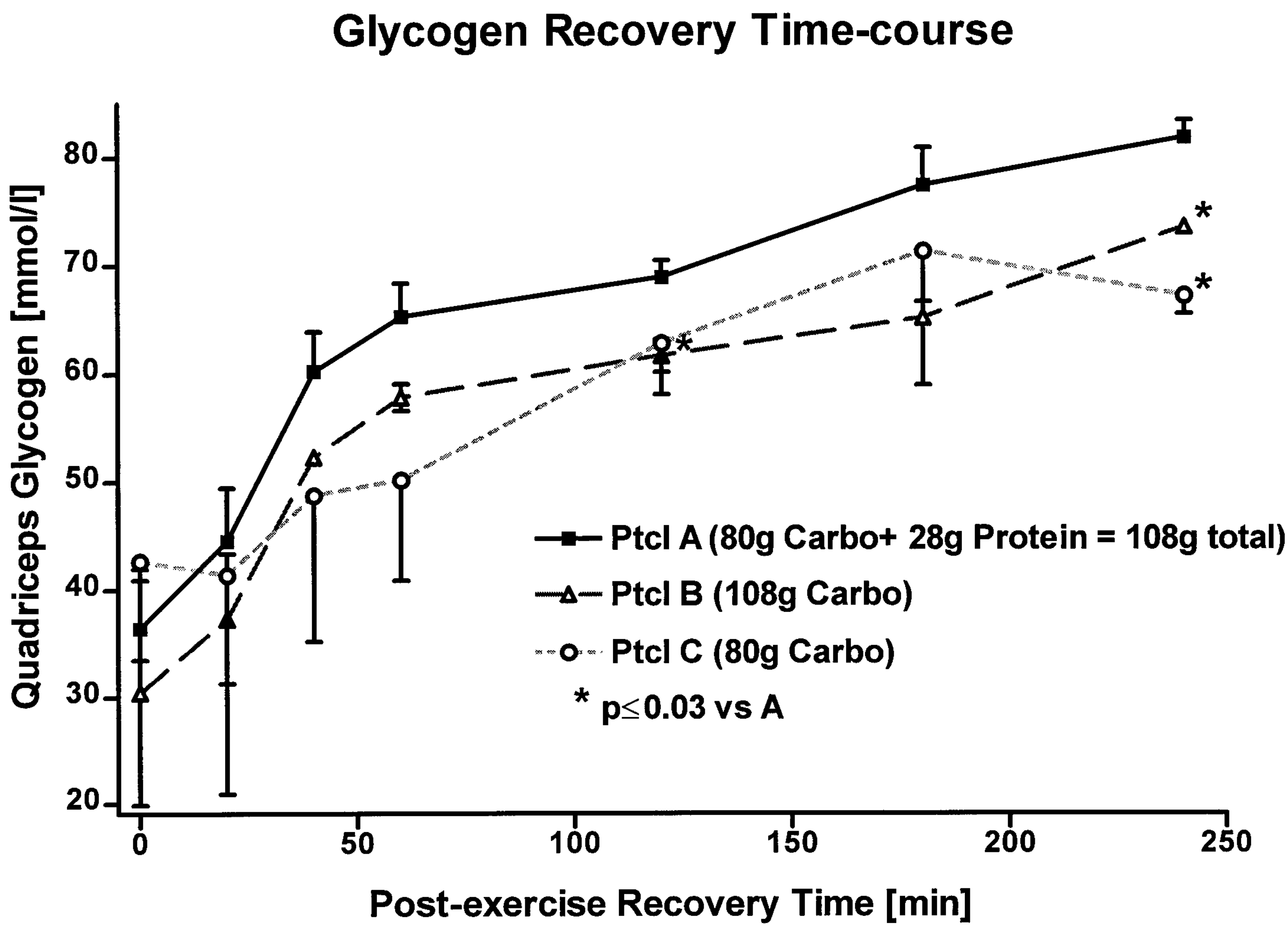

0.20
0.15
0.10
0.05
0.00
Net Liver Glycogen Depletion
[mmol/l-min]
Male
Female-Luteal
Female-Follicular
Study Group

Figure 8a:


Glycogen Recovery Time-course
Quadriceps Glycogen [mmol/l]
Post-exercise Recovery Time [min]
Ptcl A (80g Carbo+ 28g Protein = 108g total)
Ptcl B (108g Carbo)
Ptcl C (80g Carbo)
* p≤0.03 vs A
20
30
40
50
60
70
80
0
50
100
150
200
250

8b:

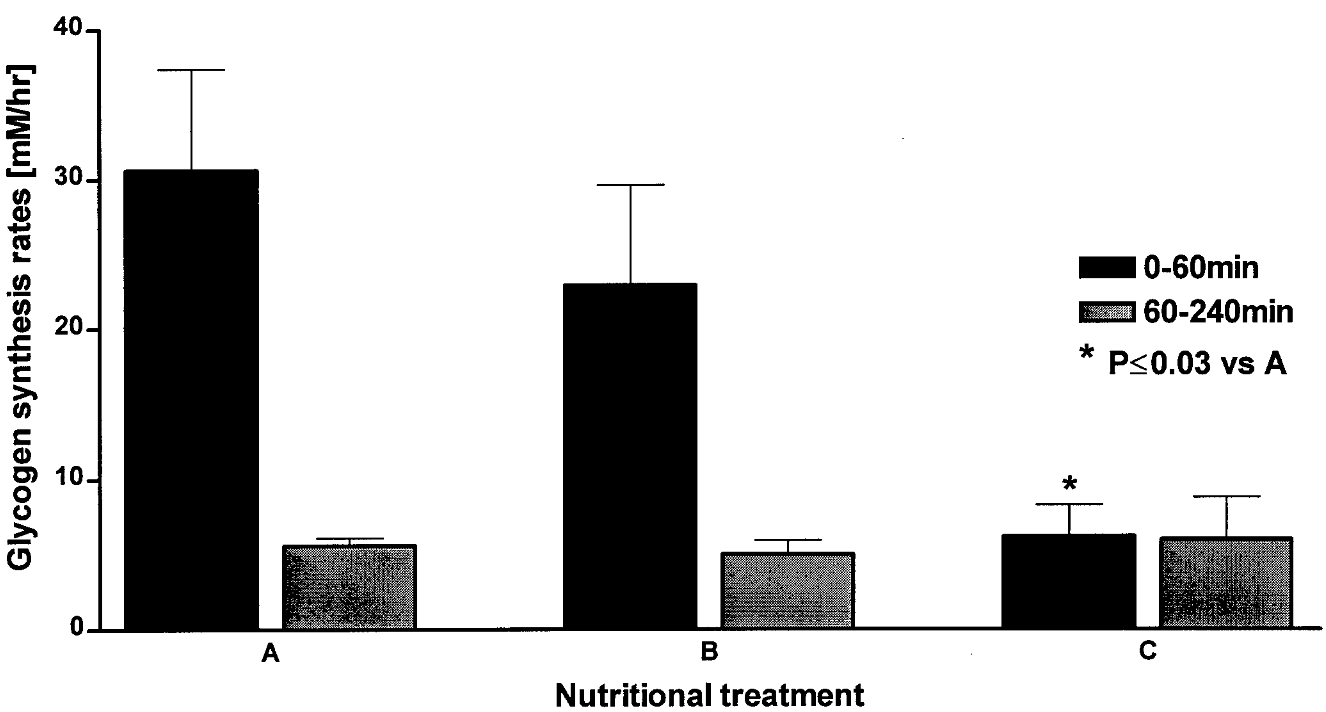
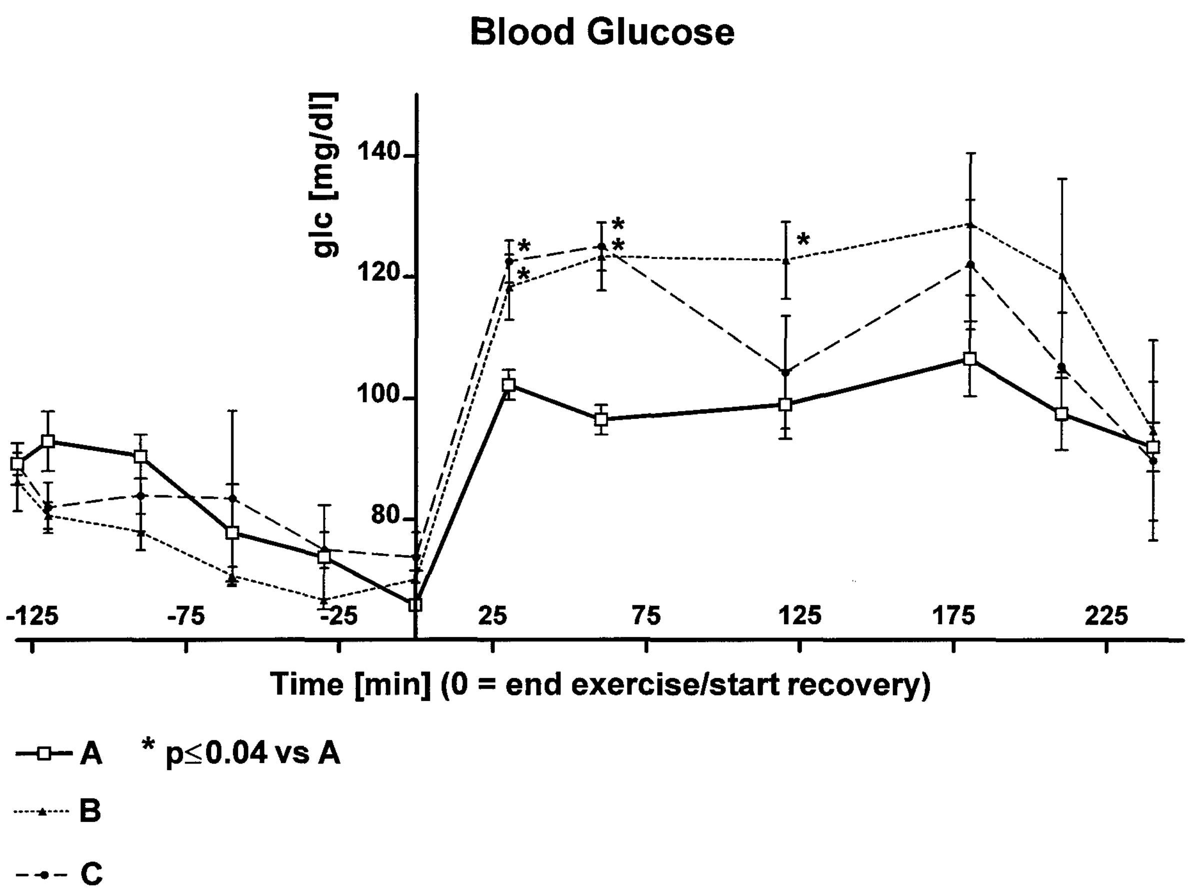

Glycogen synthesis rates [mM/hr]
Nutritional treatment
0-60min
60-240min
* P≤0.03 vs A
0
10
20
30
40
A
B
C

Figure 9:


Blood Glucose
glc [mg/dl]
140
120
100
80
-125
-75
-25
25
75
125
175
225
*
Time [min] (0 = end exercise/start recovery)
A
* p≤0.04 vs A
B
C

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 28 July 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Phyllis Rinehart

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
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